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[COMMUNICATION]

# Interspecific Transplantation of Developing Tissues and Their Subsequent Differentiation in Flies

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**ABSTRACT**—Imaginal leg discs from the larvae of housefly, *Musca domestica* were cultured in the body cavity of the pupae of fleshfly, *Sarcophaga bullata*. The implanted discs were not rejected by the foreign species. On the contrary, they differentiated fully and completed metamorphosis in time according to their own developmental program. However, the tanning and hardening of the cuticle occurred along with that of the host after eclosion of the host fly. These developmental events are discussed in relation to the hormonal milieu of the host species.

## INTRODUCTION

During the course of their development flies go through distinct stages such as larva and pupa before metamorphosing into adults. Several adult structures like the legs, wings, eyes, etc., differentiate in the pupal stage from groups of embryonic cells called imaginal discs. Simultaneously, the central nervous system (CNS) too undergoes considerable reorganization and establishes specific neural connections with the newly formed peripheral target tissues. A great deal of information is available with regard to the formation of specific nerve connections between the CNS and target tissues of the same species such as crickets [1], fleshflies [2] and fruitflies [3]. However, very little is known whether this specificity is restricted within a species or it extends beyond species boundaries. To explore this aspect of neuron-target interaction, developing tissues of the housefly, *Musca domestica*, were cultured into the pupal body cavity of the fleshfly, *Sarcophaga bullata*, and this report describes the metamorphosis of such transplanted imaginal discs. The differentiation of transplanted CNS of the housefly has been reported elsewhere [4].

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## MATERIALS AND METHODS

The housefly, *Musca domestica*, and the fleshfly, *Sarcophaga bullata*, were cultured in the laboratory under constant conditions of temperature (25°C) and photoperiod (16L:8D). The housefly larvae were raised in an artificial diet containing milkpowder, wheat bran and sawdust, while the fleshfly maggots were fed with fresh beef liver.

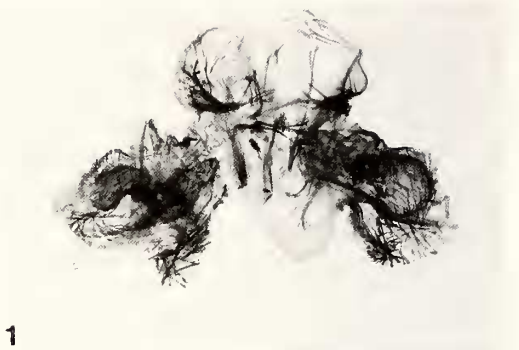
Since the housefly is smaller in size with a shorter pupal life they were used as donors, while the larger fleshfly with longer pupal period served as hosts. The imaginal leg discs of mature 3rd instar larvae of *Musca domestica* were dissected in insect saline and implanted into freshly formed fleshfly prepupae. The transplantation method was based on the technique of Bhaskaran and Sivasubramanian [5] but slightly modified as described in Sivasubramanian and Nassel [2]. Of the total of 76 successful implants, 36 were recovered 6 days after the operation from the host pupa (total pupal period of donors) and 40 were recovered 12 days post operation after eclosion of the metamorphosed host flies. The tissues were examined as whole mounts.

## RESULTS AND DISCUSSION

Housefly, the donor, is about half the size of the fleshfly, the host. The imaginal leg discs of the housefly being very small (0.55 mm long; 0.38 mm wide) compared to the volume of the host pupa (170 $mm^3$) several discs could be cultured in the same host. Accordingly, 2–4 discs from housefly larvae were transplanted into the fleshfly pupae. The time taken to complete metamorphosis is also correspondingly shorter for housefly, i.e., 6 days as compared to 12 days for fleshfly. Therefore, the implanted leg discs were recovered from hosts 6 days after operation. As seen in Figure 1 the discs had fully metamorphosed in 6 days with well tanned bristles albeit in an uneverted condition because of their development inside the body cavity. However, the cuticle was still untanned. Figure 2 shows the metamorphosed leg discs recovered from an eclosed host fly (12 days post-operation). The cuticle of these legs were fully tanned.

Although insect imaginal discs are routinely cultured *in vitro* for understanding of hormonal control of growth [6], eversion [7], biochemistry of developmental events [8], etc., *in vivo* culture is the preferred method of developmental biologists looking into the aspects of determination [9], pattern formation [10], axonal projection [2, 11] etc. In the latter method, the discs from larval stages are transplanted into the metamorphosing stages (pupa) of the same species and examined at the completion of metamorphosis of the host. In this procedure, one of the limiting factors is the volume of the host which restricts the size and number of discs that can be cultured. This problem was solved as reported in this communication by using a larger species as host and a smaller one as a donor. The housefly discs not only survive but also complete their differentiation within the body cavity of fleshfly pupa.

Molting hormone ecdysone is an essential requirement for differentiation of imaginal discs, and, according to Wentworth *et al.* [12], there is an ecdysone peak in the host *Sarcophaga bullata* at the time of transplantation. Therefore, it is not surprising that the housefly discs complete metamorphosis within fleshfly pupae. Nevertheless, it is



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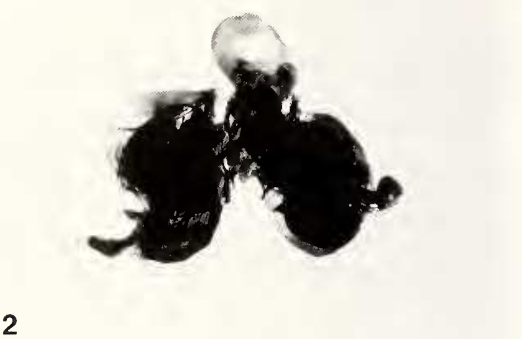

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FIG. 1. Metamorphosed leg discs. Two prothoracic leg discs recovered 6 days after transplantation showing fully tanned bristles. ×35.

FIG. 2. Metamorphosed leg discs. Two prothoracic leg discs recovered from eclosed host flies 12 days after transplantation showing fully tanned cuticle. ×35.

interesting to find that the transplanted discs follow their own inherent developmental time table to complete differentiation. That is, within a period of 6 days they were fully differentiated with well tanned bristles whereas bristle tanning begins much later, 10 days after pupariation in the host species [13]. However, the cuticular tanning of the implants occurs simultaneously with that of the host cuticle (Fig. 2). Hardening and tanning of the cuticle is a critically timed event that is controlled by the neurohormone bursicon secreted soon after eclosion of the host fly [14] and therefore the housefly legs too undergo tanning after the eclosion of the host fly.

Thus, the *Sarcophaga* pupal body cavity acts as a suitable environment for the differentiation of housefly imaginal discs. The central nervous

system of larval housefly also undergoes complete metamorphic reorganization within the fleshfly pupa [4]. Such a system has the potential to be exploited for studies of neuronal specificity by means of *in vivo* culture of the CNS with the discs of the same or different species.

## ACKNOWLEDGMENT

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